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Regeneration of Teeth and Periodental Tissues Using Stem Cells

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Regeneration of Teeth and Periodontal Tissues using Stem Cells

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Abstract

The purpose of this thesis is to serve as systematic literature review that provides a background on regenerative therapy in dentistry, as well as proposing the need for continued research. Regenerative dentistry has great potential for stem cell use. Stem cells of many types have the capability of self-renewal and differentiation into multiple cell types. With stem cell-based regeneration, dentistry can grow exponentially with the implementation whole-tooth replacement, pulp regeneration, periodontium regeneration, enamel regeneration, and dentin regeneration. Furthermore, utilizing various biomaterial scaffolds and inducing growth factor activity has been demonstrated to aid in regenerative efforts. Issues of regenerative therapies must also be considered. Once all methods, techniques, and issues have been developed and resolved, the future implications of regenerative dentistry may then be considered. There currently appears to be great promise in regenerative dentistry. Before the field can make an official emergence, more research and studies must be conducted.

I. Introduction

As it was understood many years ago, structural regeneration was merely a plausible theory. Ever since the presence and physiology of stem cells has become known, clinically-induced regeneration has been at the forefront of the minds of many researchers for the possibility of therapeutic uses. Complete understanding of how tissues undergo regeneration will shed light on how these tissues can be regenerated in a clinical setting as part of a treatment plan. This understanding includes an understanding of cell physiology and tissue development.

Stem cell physiology goes deep into the functions and mechanisms of the cellular activities of stem cells, and how they play a crucial role in regenerative therapy. There are several stem cell lineages that differentiate into varying cell types. For example: in order to generate dentin, odontoblasts must first be present. Only certain types of stem cells are capable of differentiating into odontoblasts. Understanding this will help researchers and clinicians to make the right decisions in selecting the right cell cultures for regenerative therapy in dentistry.

Developmental biology play a major role in how various tissues regenerate. Mesenchymal cells (present in developing embryos) that originate from the endodermal, mesodermal, and ectodermal layers of the gastrula will proliferate and differentiate into various cell lineages that will determine what types of cells will develop. As these cells are further developed, they continue to proliferate and begin to form specific tissues. In regenerative therapy, structures are undergoing development all over again, just as they once did during gestation. Having this knowledge will give way to the fundamentals of undergoing regenerative therapy.

Upon the establishment of all reviewed and verified methods for regenerative therapy, there will be a different outlook for patient treatment and healthcare as a whole. For the remainder of this paper, the field of dentistry will be the primary focus of regenerative therapy. Even though further research is required and human clinical trials have yet to begin, regenerative therapy in dentistry is very promising.

Current Pathological Issues in Dentistry

Today, there are many reasons to visit the dentist. Some are for cosmetic reasons or preventative reasons, but the majority of visits to a dentist are related to problems such as tooth decay and gum disease. Both of these issues contribute directly to the eventual loss of teeth. Since teeth play a major role in our ability to live healthy lives, patients without teeth may begin to exhibit malnourishment. This is because they are not able to consume normal amounts of food.

The human dentition is composed of four types of teeth: incisors, canines (cuspids), premolars (bicuspids), and molars (tricuspids).The primary function of the incisors and canines is to grasp, rip, and tear food, while the primary function of the premolars and molars is to crush and grind food. Patients that are missing any of these teeth will exhibit impaired mastication (chewing). Teeth also function in aiding with speech. Certain speech impediments can become very common in patients with missing incisors. Furthermore, the complete absence of teeth (edentulism), is attributed to a sunken appearance of the face; therefore, tooth loss not only has functional repercussions, but also esthetic repercussions.

The most common pathological issues in dentistry today are dental caries and tooth decay, gingivitis, periodontal (gum) disease, missing teeth, malocclusions, impacted teeth, and oral cancers (VCU School of Dentistry). All of these issues are treatable and preventable. There are some exceptions for conditions that are treatable, but not entirely preventable, as some conditions, such as impacted wisdom teeth or oral cancer, may arise independently of the patient's lifestyle.

Common Dental Treatments

This thesis will focus on common dental treatments for missing teeth, infected and decaying teeth, and infected periodontal tissues. To address tooth cavities (dental caries), high-speed and low-speed hand pieces are used with a wide variety of drill bits to drill into the site of infection of the tooth as part of the preparation of the tooth. Because the majority of caries occur within the grooves of molars and premolars, the tooth is prepared by drilling along the central groove of the tooth and in between the individual cusps. Some dentists describe the prepared shape as a "gingerbread man" as shown below in Figure 1.

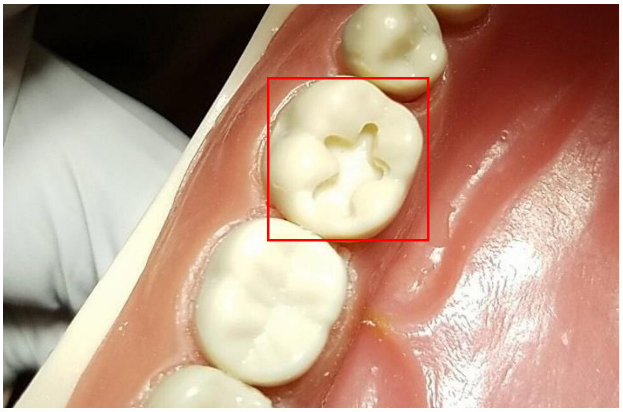


Figure 1. Image credit: Joshua A. McKoy

Once the above shape has been achieved, composite restorations or amalgam restorations can be completed. The purpose of this procedure is to remove the infected part of the tooth as well as some of the dentin so that various materials can be placed into the preparation site, and then, adjusted to achieve the original anatomical features. This approach simply serves as a patch for the tooth following the emergence of a cavity (VCU School of Dentistry).

In instances where the infectious bacteria (most often, *Streptococcus mutans*) responsible for dental caries has progressed through the enamel and dentin and breached the pulp cavity of the infected tooth, root canal therapy is required. The root canal therapy, also known as, endodontic treatment, is another common dental treatment procedure. The basic methods of this procedure are to drill into the tooth and widen the site of the initial infection of the tooth crown and then continue to drill until the pulp cavity is reached. Using various endodontic instruments, dentists clear out the infected pulp cavity until no pulp remains, and then sterilize, fill, and seal the damaged area (VCU School of Dentistry). The purpose of this procedure is to remove all infected tissues from the tooth, including the pulp. Once bacteria reach the pulp, the infection spreads very rapidly. If left unchecked, the infection could spread into the jaw and surrounding tissues. At this point, it has become a very serious issue, and the danger of a systemic infection presents itself. Endodontic treatment prevents infection from progressing further if a simple filling is no longer an option.

Another chronic disease that is very common is periodontitis. This condition progresses from gingivitis, which is the inflammation of the gums. Periodontitis is the inflammation and infection of the periodontal tissues that support the tooth. These infected tissues may include the cementum, periodontal ligament, gingiva, and alveolar bone. The infection of any of these tissues can be very serious problem. If left unchecked, not only will the tooth no longer be able to remain in its socket, but the infection could also spread.

In many cases, because periodontitis is preceded by gingivitis, common treatments primarily include improving the patient's oral hygiene. The hygienic measures include: better brushing of teeth, flossing, and deep cleanings. In more progressed cases, anti-inflammatory medications and antibiotics (like doxycycline) may need to be administered. There are, however, many cases of periodontitis that are so severe that surgery is required. A few periodontal treatments include: bone grafts; gum grafts; ridge augmentation; dental implants; crown lengthening; and scaling and planning. Again, for the purposes of this paper, the surgical treatments in periodontology will be discussed further.

There are a multitude of dental treatments that address functional and esthetic concerns of multiple missing teeth. The most common treatment for this issue is the use of dentures. Dentures are usually associated with geriatric patients, but there are cases in which younger patients receive dentures. There are also two main types of dentures: full dentures and partial dentures. Full dentures are prosthetic devices used to resemble the upper and/or lower arch of the human dentition. Dentures include prosthetic teeth and gums. For patients to receive dentures, they must first be fitted by getting an impression of the patient's upper and/or lower arch. Here, the size of the denture and the teeth within in are very important factors to consider. The dentist must ensure that the denture is comfortable and fits on the buccal (cheek), lingual (tongue), occlusal, and labial (lips) aspects of the patient's mouth. It is also important to ensure that no undue strain is being forced on the patient's jaw at and around the temporomandibular joint (TMJ).

A common dental procedure that can even be seen in many different dental settings, specialist practices and general dentistry practices alike, is the implantation of artificial teeth in place of a lost or removed tooth into the maxilla (upper jaw bone) or into the mandible (lower jaw bone). The purpose of receiving a dental implant is to replace a tooth that has been removed or has just fallen out due to disease. Dental implants are put into place following a series of drilling into the bone where the dentist has determined the implanted tooth should be placed. Once the implant is placed into the bone, the socket is sutured and sealed for it to heal and for osseointegration to take place. After enough time has passed, the patient will return and will be inspected. If the implant is stable and in good condition, an abutment is placed into the implant, which is where the crown of the artificial tooth will be placed. Figure 2 illustrates this procedure:

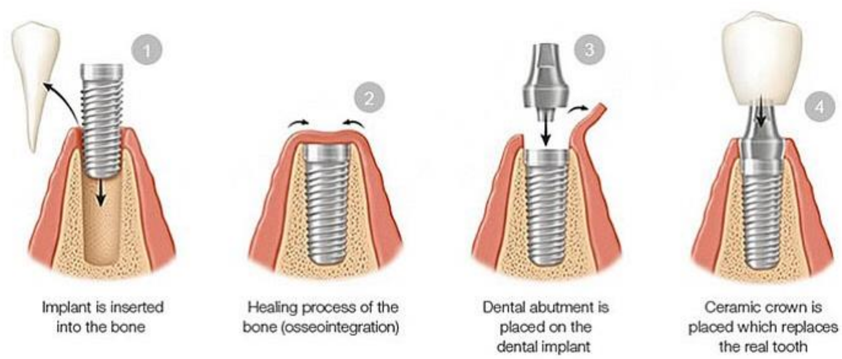


Figure 2. Image credit: dCourier.com

All of these common dental treatments will be discussed throughout this thesis, but more emphasis and discussion will be centered on the use of dentures and implants in comparison to the use of stem cells in regenerative dentistry. The purpose of this discussion is to be able to delineate the characteristics and uses of a common removable prosthodontic treatment and implants to show weaknesses of dental care today in order to emphasize the importance of continued research to lead to the development and implementation of regenerative dentistry.

Shortfalls of Current Dental Treatments

Each of these common dental treatments have pros and cons. From a business standpoint, patients are considered to be consumers of dental healthcare services. It is the duty of the consumer to make informed decisions. These decisions should be made based upon ample knowledge of the benefits of certain services, as well as their drawbacks. Because dentistry is an extremely complex field, it is the duty of the dentist to inform his/her patients of what situation they are in, as well as what approaches should be made. Some treatments may be better than others for a plethora of varying reasons. The benefits of these treatments and procedures are well-established, but their drawbacks will be one of the focuses of this thesis. It is also important to note the limitations of these treatments, because where these fall short, regenerative therapies, with the use of stem cells, may be able to pick up the slack. For the purposes of what is being examined in this paper, the drawbacks of the use of dentures, implants, endodontic treatment, and periodontic treatment must be properly understood.

Currently, there is a strong demand and necessity for the use of and practice of dental implantology. While it is still rather a new field within dentistry, it has really captivated the minds of patients and dentists alike. Implants not only fill the void of not having a tooth (or teeth), and the nutritional and esthetic repercussions associated with it, but dental implants also solve the main problem of rapid bone resorption. There are many benefits to having dental implants, but there are also drawbacks and problems that are not always evident. The major issue is the possibility of silent inflammation. Although titanium is a good material to use for osseointegration, titanium particles may be to blame for inflammatory reactions surrounding the implant. One study suggests that titanium implants lead to peri-implantitis, which can result in fatty degenerative osteonecrosis of the jaw. This inflammatory condition is accompanied by TNF-α, and the R/C cytokines, RANTES/CCL5. These cytokines have been linked to other inflammatory diseases of the body, which may all be indirectly caused by T-IMPs. Unfortunately, this may initially go unnoticed (silent inflammation) (Lechner, et al). This concept is depicted in Figure 3 below.

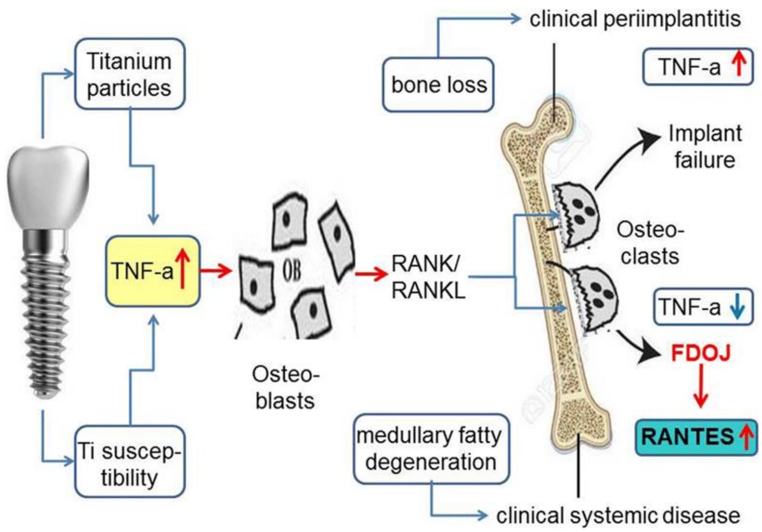


Figure 3. Image credit: Lechner, et al.

Endodontic root canal treatment, also simply known as a root canal, is absolutely necessary in the current state of the field of dentistry. It is capable of saving a tooth after its pulp canal has become infected or inflamed following the progression of an initial cavity or injury. Although root canals are largely capable of beneficial results, root canals are not perfect. Although no procedure is perfect, but when compared to the possibilities of regenerative dentistry, one may be subjectively perfect (regenerative therapy). Root canal treatment can be the ideal option when patients are faced with other options such as receiving implants, bridges, or dentures, all of which first require extraction.

Endodontic treatment is still highly preferred due to the fact that the original tooth is still present. As time has progressed, endodontic technology and methodology have improved. However, there are still issues with the endodontic treatments of today.

The main problems with root canal treatment is that once the procedure is complete, the tooth is completely devoid of its pulp. Dental pulp is the soft tissue on the interior of the tooth. It is made up of blood vessels, nerves, and soft tissue. If the pulp is missing, the tooth will have no way of “communicating” to the patient that an injury or infection has occurred. If an infection reaches all the way through the tooth, an abscess can form, thus leading to the surrounding bone becoming infected. Infections of the jaw bone turn into very serious cases. And when left unchecked, these infections can continue to spread throughout the body. There are many cases in which unchecked tooth infections led to death. One such case was that of 12-year old, Deamonte Driver, who died in 2007 of a brain infection that started from a tooth infection (Owings, ABC News). Cases like these would become more prevalent if it were not for the pain of a toothache that we all feel.

Two other issues that root canals can cause are brittle teeth and thinner pulp chamber walls (Chen, et al). The occurrence of brittle teeth comes from the removal of all dental pulp from the carious/injured tooth. The pulp provides nourishment to the tooth, and without the pulp, the tooth will become malnourished and brittle. This brittleness can easily result in repeated caries and injuries. It is important for teeth to have pulp chamber walls that are thick enough (Chen, et al). Before gutta-percha is placed into the canals and the tooth is filled with a temporary filling, the entire pulp chamber must be cleaned and shaped (VCU School of Dentistry). During the cleaning and shaping of the pulp chamber, some dentin is lost as a result of the use of different endodontic instruments. The loss of dentin can result in chamber walls that are too thin, which can lead to other pathological issues.

Periodontal treatment is essential for the preservation of the periodontium. As previously mentioned, the periodontal tissues include cementum, periodontal ligament, alveolar bone, and gingiva. The main drawback with periodontal treatment is that in order to treat and fix problems of the periodontium, in some surgical cases, tissues that have been severely damaged have the potential of being left in conditions that are less than ideal. Therefore, if infection develops in the periodontal tissues directly adjacent to the root of the tooth and the alveolar bone becomes severely necrotic, the lost bone cannot be regrown on its own. If the osteonecrosis was severe enough, a bone graft may be required. As a result, more surgical measures must be taken, as well as the risk of possible bone graft failure. The bone graft fails when the porcine or bovine ground bone fails to integrate with the human alveolar bone.

While dentures are very simple to use and care of, from a functional standpoint, there are underlying issues that may come about with the use of dentures that dentists and patients, alike, must be aware of. With age, the body becomes weaker, skin becomes less elastic, and bones become more brittle. Continued mechanical stresses on bones help to keep bones healthy and strong because when bones are not being stressed or stimulated, osteoclast activity increases, which results in bone resorption (Kennestone Periodontics). Osteoporosis can also result from increased brittleness of bone. These issues are relevant to dentistry because when teeth are lost and not replaced, the bone that once supported the tooth will begin to resorb (Kennestone Periodontics). Once one tooth is lost, other teeth will begin to shift positions in the mouth and are at increased risk of being lost. If the lost teeth are not replaced, other problems will inevitably arise.

As it is highlighted in this paper, there are three main problems with dentures. One problem is dentures only take care of esthetic issues, such as large gaps in the smile due to missing teeth, and nutritional issues, such as not being able to chew and eat. The second problem is that dentures do not stimulate the underlying maxillary bone and mandibular bone. As mentioned previously, these bones require mechanical stress in order to maintain their shape and overall integrity. Dentures are not able to mimic the normal mechanical stresses that teeth should place on bones. It is for this reason that it is often preferred for patients to receive dental implants instead of only receiving dentures. Dental implants provide the mechanical stress that the surround bone requires. Another issue related to bone resorption is the progression of facial deformities, such as a sunken look in the lower aspect of the face. This appearance can be observed very often in geriatric patients. To those with a slightly trained eye, this facial appearance can be easily recognized as the result of missing teeth. This issue also has psychosocial impacts associated with it. The third issue with the use of dentures, is the occurrence of denture stomatitis, which is the inflammation of gingiva and palate of the oral cavity (Yarborough, et al).

Even with these drawbacks of common dental treatments, these approaches should not be completely disregarded as they do not substantially hinder the practice of dentistry. However, the significant limitations of these therapies necessitate further research into potential regenerative therapies that could restore native tissues to better address dental pathologies.

Proposition for Using Stem Cells in Dental Treatment

Based upon common dental pathological issues, common dental treatments, the shortfalls of these treatments, and the current state of the field of dentistry, this thesis will serve not only as a systematic review of literature, but also a proposition for the development and implementation of regenerative dentistry. The following sections will discuss regenerative dentistry and possible methods of regenerative therapies. If these regenerative therapies are to be used, the biological approach and clinical implications must be understood. The discussion of the clinical implications will include the practicality of regenerative therapy in dentistry, economic considerations, and future implications for dentistry as stem cells are concerned will also be expounded upon.

II. Stem Cells

Stem cells are undifferentiated cells capable of cell division and proliferation. They replace differentiated adult somatic cells, thus enabling them to be able to maintain the balance between cell death and cell renewal (Gilbert, pgs. 319-320). When stem cells divide to produce two daughter cells, one cell remains undifferentiated as another stem cell (self-renewal), while the other daughter cell differentiates. Based upon the stem cell concept, stem cells may have differing circumstances in which the types of daughter cells remain consistent (Gilbert). In single-cell asymmetry, as outlined above, one daughter cells used for self-renewal and the other for commitment to differentiation. This keeps the cells balanced in that for every division, one cell is undifferentiated, while the other is differentiated. Population asymmetry refers to both daughter cells being committed to differentiation. This also keeps the cells balance in that if a greater need of committed cells are needed, both daughter cells will differentiate. As a result, other stem cells will produce two undifferentiated daughter cells. Both tenets of the stem cell concept help maintain the balance in the types of stem cells that are present (Gilbert, pgs. 319-320).

Within the adult stem cell lineage, multipotent stem cells give rise to committed cells, which lead to progenitor (transit amplifying) cells, and lastly, differentiated cell. This concept is most commonly seen with emergence of erythrocytes (red blood cells), megakaryocytes (which fragment to form blood platelets), and lymphocytes (white blood cells) all from one common hematopoietic stem cell (Gilbert, pgs. 319-320). There are many different types of stem cells. The most commonly mentioned stem cell types include: hematopoietic, epidermal, neural, hair, melanocyte, skeletal muscle, gut, germ, and tooth stem cells.

When discussing stem cells, there is a certain terminology that is used to refer to specific cells and cell types. Embryonic stem cells originate from the inner cell mass of mammalian blastocysts or from fetal gamete progenitor cells. Adult stem cells are the previously-mentioned stem cell types that are found in specific tissues, such as tooth stem cells. Potency refers to the degree of differentiation to which a stem cell is capable of committing. Totipotent stem cells are capable of forming every embryonic cell and trophoblastic cell of the placenta. Pluripotent stem cells are capable of forming all embryonic cells, but not placental cells. Multipotent stem cells can form either embryonic stem cells, or just adult cells. Here, commitment is limited to a specific subset of cell types. Lastly, unipotent stem cells are found only in specific tissues, and are used only for regeneration of a specific cell type. Progenitor cells, also referred to as transit amplifying cells, are related to stem cells, but are not capable of unlimited self-renewal. After dividing a certain number of times, progenitor cells will differentiate. Additionally, progenitor cells are called transit amplifying cells, because they will usually divide as they migrate from their stem cell niche. A stem cell niche is a regulatory microenvironment that allows for controlled self-renewal, differentiation, and regeneration. Stem cell niche examples include, mesenchymal stem cells in bone marrow, adipose, cardiac, placental, and umbilical cord tissues. Skeletal muscle stem cells also have niches between the basal lamina and muscle fibers (Gilbert, pgs. 319-323).

Unique stem cell niches exist in different organisms - even organisms that are closely related. Through evolution, some animals may have lost some stem cell niches, while others retained them. An example of this are the lingual and labial stem cell niches of mouse incisors. The presence of these niches in mice allow their teeth to grow and regenerate throughout their lifetimes. This is not innately possible for humans, because like many other animals, humans have lost this stem cell niche (Gilbert, pg. 326).

III. Models for Regenerative Therapy

So far, the necessity for regenerative dentistry has been described through a discussion of the limitations of specific aspects of dentistry. Although, these limitations do not greatly hinder modern dental practices, these limitations to provide the evidence for the need for better treatment methods. As will be discussed later, it is the moral and ethical responsibility of the dentist to do no harm (ADA). This is not always possible with all of the current treatments in healthcare. If it is possible to treat and help patients even better while doing less harm, this possibility must be further explored.

There are a number of methods that entail the regeneration of dental tissues. Many of these methods are comprised of the isolation and cultivation of various types of stem cells, or just the activation of different developmental processes that involve the use of stem cells. Before delving deeper into how different methods of dental regeneration (and their mechanism) can occur, the proposed models for regenerative therapy will be discussed in great detail first.

Whole-Tooth Replacement

In the event of severe tooth infections, in which the tooth cannot be saved, the tooth is extracted, and a socket remains. Similarly, in the event of injury, such as blunt force trauma that can cause the tooth to be forcibly removed from its socket, there is an empty space left behind. If the tooth was extracted following an infection, many times, a dental implant will replace the tooth. A recommended, the tooth can possibly be saved and placed back in its original socket, in the event of trauma. Careful care must be taken here. Without touching the root of the tooth, the tooth can be placed back into its socket, and professional help should immediately sought. If this isn’t possible, the tooth can be placed in the mouth beside the cheek until dental assistance is received - within 30 minutes (VCU School of Dentistry). If the tooth cannot be saved, a dental implant may replace the tooth (to prevent bone loss). These are the common measures that are taken to replace a tooth after a tooth socket has been vacated. If the original tooth cannot be saved, an alternate method for whole-tooth replacement should be explored.

As is the case with other organs, teeth can be formed completely in-vitro (Oshima, et al). This is only possible with the use of stem cells, of course. In normal developmental biology, organogenesis occurs through the epithelial-mesenchymal interaction in the developing human embryo. This process has been able to be replicated in order to reproduce entire organs (Oshima, et al).

There are two main methods by which whole tooth-replacement has not only been proposed, but demonstrated. Tooth replacement through regeneration has been performed through functional tooth regeneration, and functional tooth replacement (Oshima, et al). What functional tooth replacement means here is, through the implantation of a functional tooth unit into the empty tooth socket, followed by osseointegration with the surrounding alveolar bone, the original tooth has be replaced, and function has been restored. Both general methods are illustrated in Figure 4.

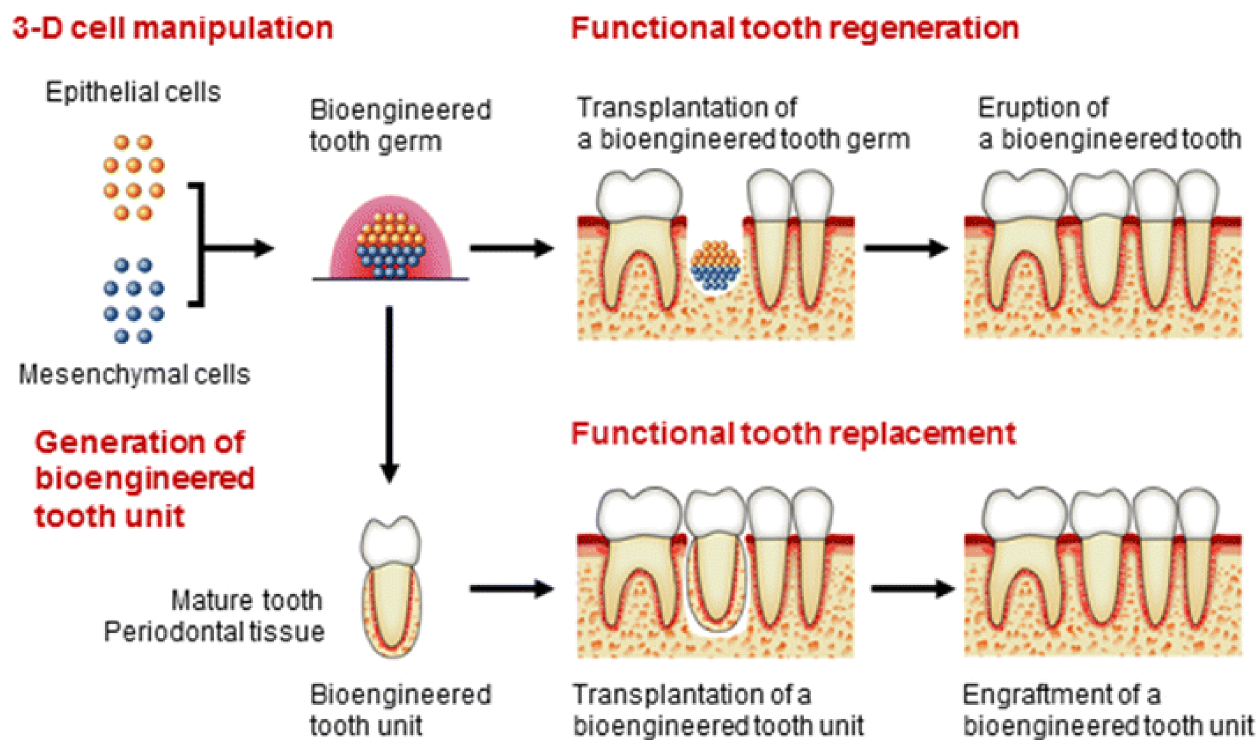


Figure 4. Image credit: Oshima, et al.

Functional tooth regeneration can be undergone in two similar ways. Because mesenchymal cells engage in a series of cellular signaling processes in the development of a tooth germ in developing embryos along with the developing oral epithelium, both ways involve this being accomplished with the use of a natural bioengineered tooth germs. One method uses mesenchyme that originated from dental stem cells, while the other uses mesenchyme originating from non-dental stem cells. The non-dental stem cells included: embryonic stem cells, neural stem cells, and adult bone-marrow-derived stem cells (Ohazama, et al). In a study where teeth were regenerated in mice as desired, the importance of using non-dental stem cells was to, 1) ensure that the non-dental mesenchyme would express odontogenic genes when interacting with oral epithelia just like dental mesenchyme would, and 2) to demonstrate that stem cells of different types can be used to regenerate whole teeth (Ohazama, et al).

The functional tooth replacement method has been demonstrated, and proven effective in mice (Oshima, et al). As depicted in Figure 5a, once a tooth has been lost, the bony socket will be prepped for engraftment. Once the tooth unit was transplanted, the periodontal tissues of the engrafted tooth unit were able to integrate with the surrounding bone which it was placed into (Oshima, et al).

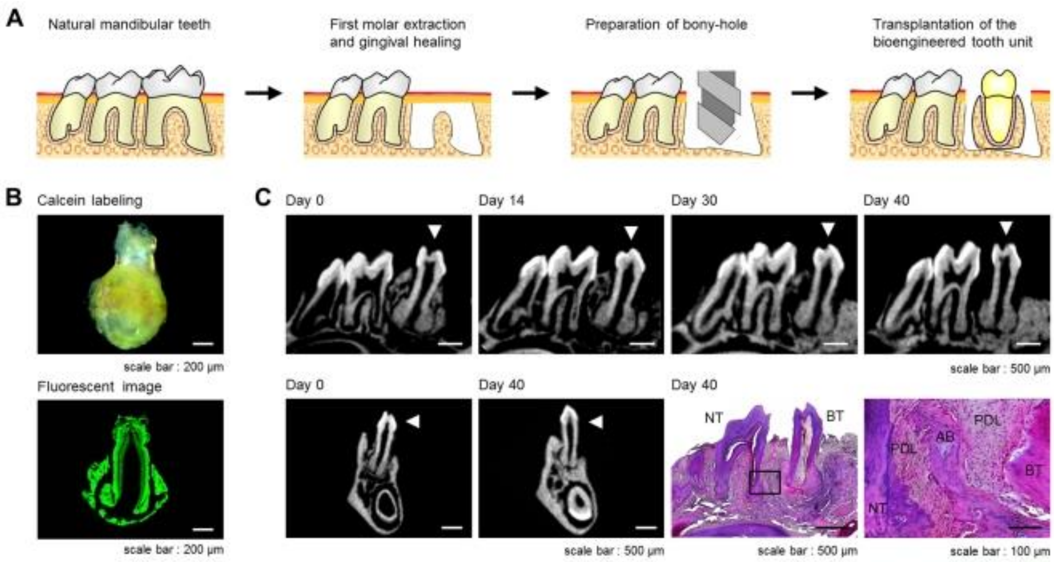


Figure 5. Image credit: Oshima, et al.

In both methods, anatomical features are restored. Additionally, physiological features must be restored. These features include: mastication (chewing), and periodontal responsiveness to noxious stimuli (Oshima, et al). Restoration of physiological features are extremely important in the regenerative dentistry. Artificial teeth, like implants, are not able to restore the tooth's original and necessary physiological functions. An example of periodontal responsiveness to mechanical stress is the tooth being able to slightly sink or give into its socket in response to pressure (Oshima, et al). This feature is not possible with dental implants. It has been demonstrated that, over time, this may lead to inflammation of the bone with which the titanium implant has integrated (Lechner, et al). This is another reason why regenerative therapy is needed in dentistry.

Pulp Regeneration

In the realm of regenerative dentistry, the capability of pulp regeneration will be another important aspect of patient treatment options. When a patient's tooth has become injured or infected to the point that the tooth's pulp has become detrimentally affected, the dentist will provide the patient with a list of treatment options. The options will often come down to the patient choosing between endodontic treatment, or having the tooth extracted. It is currently advised that patients choose the option that will save their tooth, rather than losing it. In a future where regenerative therapy is commonplace in dentistry, dental pulp regeneration may be amongst those options, and ideally, the preferred option.

In studies that were able to regenerate dental pulp in mice and swine, dental pulp stem cells (DPSCs) and stem cells of the apical papilla (SCAP) were the primary stem cell types used.) (Chen, et al; Zhu, et al). There are different ways in which regenerated dental pulp has been achieved. In all the ways described in available literature, a variety of cell carriers and scaffolds are used. The different carriers and scaffolds that were used were HyA gel, hydroxyapatite tricalcium phosphate (HA/TCP), platelet-rich fibrin (PRF), and poly-D,L-lactide & glycolide (PLG) (Zhu, et al).

In order to regenerate dental pulp, dental pulp stem cells and apical papilla stem cells must first be isolated and combined with a scaffold of which there are various types. There are two main ways in which dental pulp can be regenerated. Stem-cell/scaffold constructs can be inserted into human tooth fragments, which will be transplanted subcutaneously (Zhu, et al); or they can be inserted directly into a tooth following a pulpectomy (Chen, et al). This methodology is illustrated in Figure 6.

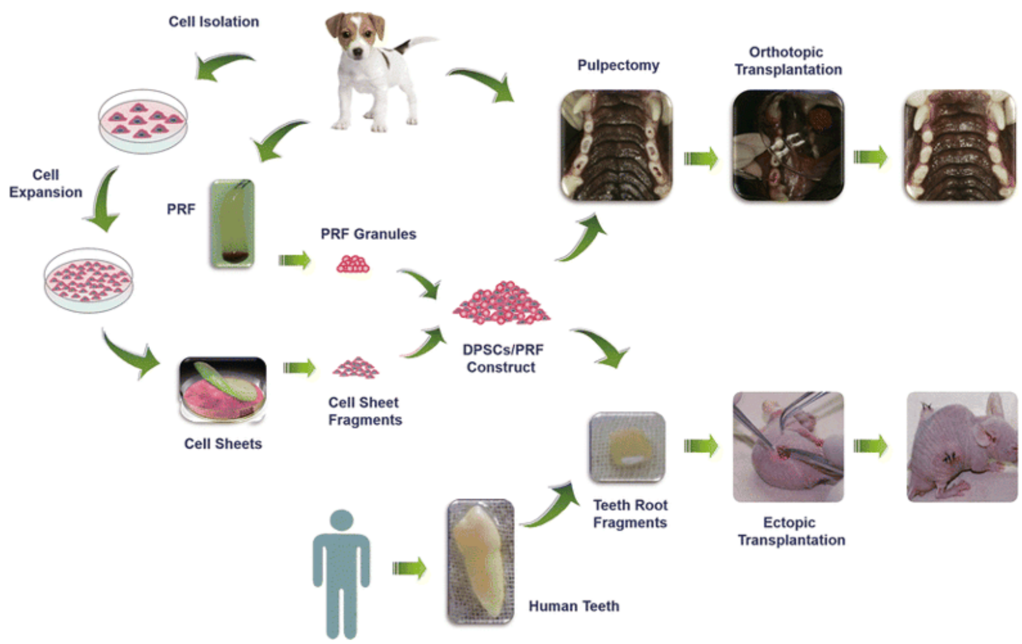


Figure 6. Image credit Chen, et al.

In recent studies, during the evaluation of regenerated structures, regenerated dentin at the pulp-dentin complex was demonstrated, as well as regenerated dental pulp (Zhu, et al). Both of these results can been seen in Figures 7 and 8.

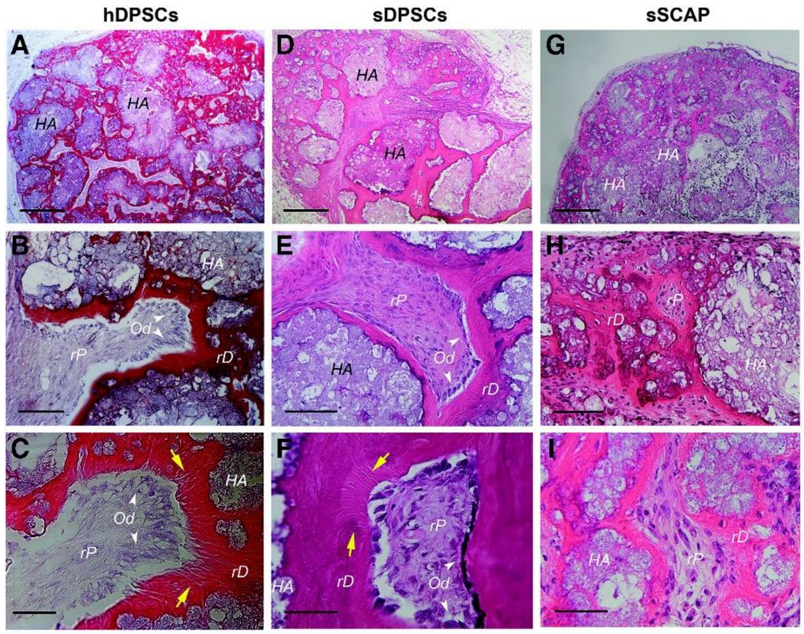


Figure 7. Image credit: Zhu, et al.

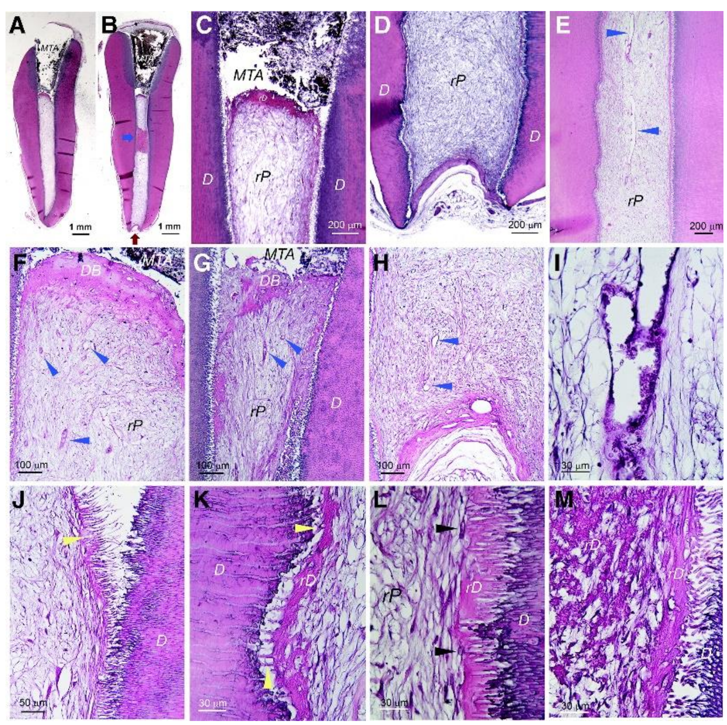


Figure 8. Image credit: Zhu, et al.

Positive results of regenerated pulp-dentin complex can be further verified by the presence of proteins produced by odontoblasts. Odontoblasts are the cells responsible for forming dentin. When odontoblastic activity is occurring, odontoblasts are known to produce the proteins: nestin; DSP; DMP1; and BSP. In studies where the dentin-pulp complex was regenerated and odontoblastic-activity was measured, high levels of these proteins were shown (Zhu, et al).

Although, human teeth have been used, the purpose of using animal models for regenerative therapy is to avoid ethical issues. It has also been preferred to use animals with similar anatomical features to humans, such as mini-swine (Zhu, et al).

Periodontal Regeneration

Also known as periodontitis, periodontal disease today is one of the most common chronic diseases that affects mankind. It can be caused by trauma to the area, but it is most often associated with bacterial infections. In cases where patients do not brush their teeth or floss well enough, a build-up of plaque can occur. An important component of dental plaque is bacteria. This bacteria, when left unchecked, can invade into the periodontal tissues. When the bacteria continue to proliferate in these tissues, the periodontium is thus compromised. As a result, the periodontal tissues are no longer able to sustain and hold the tooth. This will ultimately lead to edentulism, or tooth loss.

It has been established that approximately 20-50% of the global population has been affected by periodontal disease (Nazir). Additionally, periodontal disease has been known to be an indicator of systemic chronic diseases that occurring simultaneously. Periodontal disease has be heavily associated with occurrences of diabetes (VCU School of Dentistry).

Furthermore, periodontal disease has been shown to cause a 19% increased risk of developing heart disease, which can also equate to a 44% increase in risk in patients aged 65 years and older (Nazir). In the United States, 47.2% of adults aged 30 years and older have been found to have some form of periodontal disease, while 70.5% of adults aged 65 years and older have periodontal disease (CDC).

With the pathology and prevalence of periodontal disease, it is important that very effective and efficient measures are followed for treatment. Currently, surgical intervention is sought when periodontal disease is in an advanced stage. There have been numerous techniques developed to heal and regenerate injured and lost periodontal tissue. Current methods include: guided tissue regeneration; bone grafting; and the use of enamel matrix derivative (Tobita, et al). In terms of stem-cell-use, mesenchymal stem cells derived from bone marrow (BSCs), have been able to effectively regenerate periodontal tissues in beagle dogs and *in vitro* (Kawaguchi, et al). There have also been achievements made in the regeneration of periodontal tissues with the use of adipose-derived stem cells (ASCs) (Tobita, et al), cementoblast progenitor cells (CBs), and dental follicle progenitor cells (DFPCs) (Luan, et al). Many research studies have been conducted to investigate the ability of a variety of cells, growth factors, scaffolds, and cytokines to induce periodontal regeneration (Luan, et al).

As previously mentioned, the main components of the periodontium are: cementum, periodontal ligament, and alveolar bone. Periodontal disease deals with the damage and loss of these tissue. For the purposes of this review, a method to regenerate cementum, periodontal ligament, and alveolar bone, respectively, will be discussed.

Cementum, which is created by cementoblasts, is the layer of the tooth that lies exterior to the dentin in the tooth socket, just as enamel lies just exterior to the dentin of the crown of the tooth. Numerous growth factors and scaffolds have been used in regenerative therapy research. Brain-derived neurotrophic factor (BDNF), which plays a role in the differentiation of central and peripheral neurons, can also be found in immune cells, osteoblasts, endothelial cells, and cementoblasts. It also has the ability to induce the differentiation of these cells. One method to regenerate cementum employed the use of high-molecular-weight hyaluronic acid (HMW-HA) as a BDNF scaffold. This technique involves the surgical implantation of the HMW-HA/BDNF scaffold complex with periodontal ligament stem cells. With the inductive effects of the scaffold complex, regenerated cementum was apparent after a number of weeks (Takeda, et al).

The periodontal ligament plays a crucial role in the functionality of the periodontium of the tooth. The periodontal ligament lies between the cementum and surrounding alveolar bone. The periodontal ligament provides the tooth with sensory feedback as well as anchoring support (Takeda, et al). Failure of the periodontal ligament ultimately leads to the failure of the periodontium, and tooth loss. Regenerating this diseased structure has the possibility of saving the tooth. As mentioned before, adipose-derived stem cells (ASCs) have been used to demonstrate their regenerative capacity for periodontal structures; more specifically, periodontal ligament. In this study, ASCs were harvested from Wistar rats, and mixed with platelet-rich plasma. This mixture was implanted into the damaged periodontal layers of the rats. After eight weeks, a histological analysis led to the observation of the presence of newly-regenerated periodontal ligament-like tissue (Tobita, et al).

The alveolar bone is the bone of the mandible and maxilla that surrounds the teeth and forms ridges between teeth (alveolar ridges). It is here that, in addition to periodontal disease, malnutrition can contribute to tooth loss. An example of this would be older patients with osteoporosis and periodontal disease. There is already much research being conducted in medicine for the regeneration of bone as whole. The methods could possibly be even applied to the regeneration of alveolar bone. The method being discussed here uses dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), and dental follicle stem cells (DFSCs) in the regeneration of alveolar bone. These dental stem cells, harvested from third molars, were mixed with a bioactive glass scaffold. This scaffold provided the dental stem cells with the necessary osteoinductive environments in order to cause the cells to differentiate into alveolar bone (Raspini, et al).

It is important to note that many studies have shown that regenerated periodontium is best achieved in non-diseased tissues. This has the potential to become problematic due to the fact that the primary demand for periodontal regeneration stems from the occurrence of periodontal disease. Therefore, in order for patients to benefit the most from periodontal regenerative therapy, they must no longer be in an active diseased state. For this to occur, anti-inflammatory treatments must be used. In this case, once inflammation has been virtually eliminated in the target tissues, then regenerative therapy may commence in order to regenerate damaged or lost periodontal tissues (Luan, et al)..

Enamel Regeneration

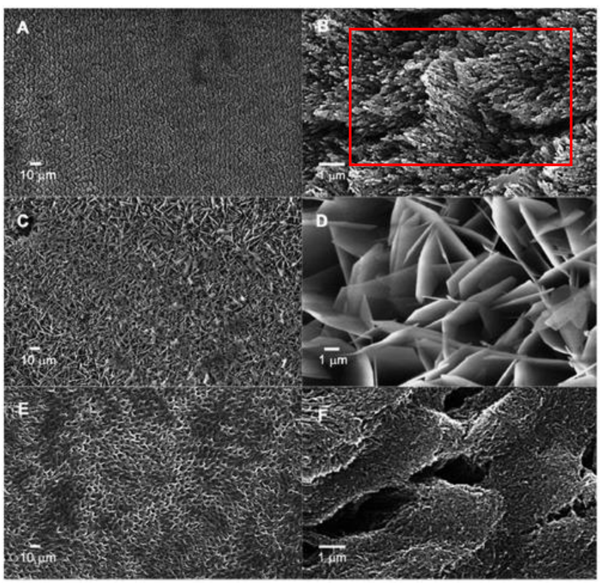
When a person smiles, the visible, white outermost portion of the tooth that is being displayed is the tooth enamel. Enamel is regarded as being the hardest material in the human body; even harder than compact bone. Enamel serves as the protective coating of the tooth.

Unlike some other structures in the body, enamel does not regrow itself. Once enamel is gone, it is gone for good. Without enamel, the tooth is greatly susceptible to infection.

There are a number of issues that can lead to the loss of tooth enamel. The most common causes are abrasions/trauma, which can come from grinding your teeth at night; acidic erosion, which is due to a diet comprised of acidic drinks and foods, such as sodas and citrus fruits; and dental caries, which result in bacteria causing demineralization of the enamel.

Currently, the main means by which tooth enamel is preserved is through the use of fluoride treatment. Fluoride ions prevent demineralization of enamel by replacing the hydroxide ions of the hydroxyapatite of enamel and converting it to fluorapatite. Fluourapatite aids in the remineralization of the enamel lesion caused by bacteria (usually *Streptococcus mutans*) (Kaplan). Fluoride can be found in tap water, tooth paste, and mouth wash rinses. The most common fluoride rinses contain solutions of fluoride and tin, but recent studies have shown that solutions containing sodium fluoride and titanium fluoride are also effective at preserving tooth enamel (de Souza, et al).

Since the whole purpose of using fluoride is to preserve the enamel, what happens when the enamel is already lost? As of right now, nothing, but there is evidence of enamel regeneration from a biomimetic approach (Kwak, et al). Unlike the other sections of this thesis, this method does not employ the use of stem cells, but rather the use of a leucine-rich amelogenin peptide (Kwak, et al). Acid-etched enamel surfaces of extracted human molars were treated with inorganic pyrophosphate (PPi) and leucine-rich amelogenin peptide (LRAP). PPi is able to control the onset and rate of enamel regenerations, while LRAP is able to regulate the orientation and shape of the growing small needle-like hydroxyapatite crystals of enamel. This experiment demonstrated that formation of dense enamel (Kwak, et al). A comparison of acid-etched enamel prior to treatment and regenerated mineral layers of acid-etched enamel can be seen in figures 9 and 10. This method has the possibility to be used in conjunction with regeneration of other dental structures with stem cells.



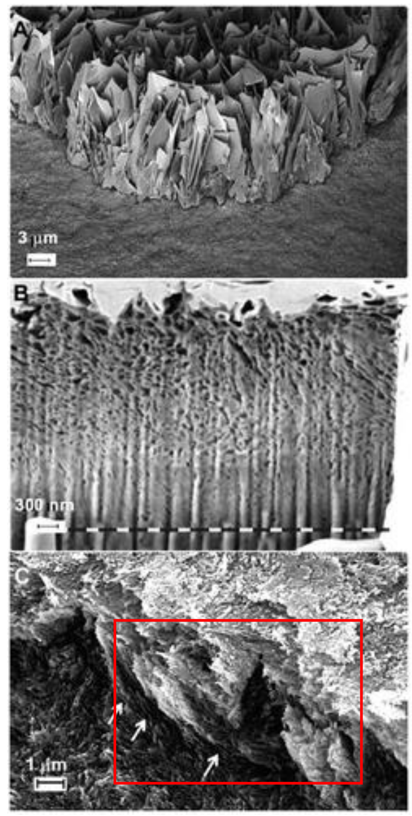


Figure 9.(Image cred: Kwak, et al) Pre-treatment

Figure 10.(Image cred: Kwak, et al)

Regenerated

<u>Dentin Regeneration</u>

Dentin comprises the majority of the tooth. It lies just beneath the enamel of the crown, and just beneath the cementum of the root. It is within the dentin where the pulp chamber is contained. While dentin is a heavily mineralized, hard tissue, it is not as hard as enamel. In the progression of dental caries, when an infection makes it past the enamel and reaches the dentin, the infection will spread much faster. This is because dentin is not as dense as enamel, and is actually somewhat porous, similar to bone. Once an infection reaches the dentin, it is urgent that dental treatment is received. Without proper treatment, the infection will progress into the dental pulp. Once the pulp has become completely infected, the tooth is practically dead, and must be extracted if endodontic treatment is not possible.

When dentin has been damaged, but ridden of all carious tissue, regeneration would be effective. Currently, superficial dental caries are treated with composite restorations and/or amalgam restorations. As mentioned earlier, artificial structures set in place to mimic and assume the function of biological structures are imperfect. If other, more feasible options were to present themselves, current restoration methodologies would not even be preferred.

Just as in the aforementioned pulp regeneration method, dentin has also been regenerated in swine. Again, the importance of using swine in research methods to be considered for human research is that swine have very similar anatomy and physiology to those of humans. This also hold true to differences and similarities between human and swine dental pulp stem cells and apical papilla stem cells, in that both are able to form colony forming unit-fibroblastic and odontogenic differentiation potential (Zhu, et al).

The regeneration of dentin-like tissues using dental pulp stem cells and apical papilla stem cells entails the use of hydrogel and HA/TCP. For *in vivo* analyses, the formation of the pulp-dentin complex, which is where the pulp of the tooth meets the wall of the chamber. In addition to this, regeneration dentin-like material was noted to be present on the surface of the HA/TCP scaffold. Furthermore, other dentinal tubules were observed. To confirm these results, the presence of nestin, DSP, DMP1, and BSP proteins were found (Zhu, et al). The presence of these proteins indicate odontoblastic activity. Odontoblasts are responsible for the formation of dentin during human development. Additionally, the presence of these proteins indicates that the injected dental stem cells were able to be properly induced into odontoblastic differentiation. This method could be used in conjunction with the regeneration of dental pulp as well.

III. Supportive Environments and Mechanisms for Regeneration

In order to achieve regeneration, not only are stem cells necessary, but so are their supportive environments. Here, biomaterial scaffolds and growth factors and their contribution to regenerative therapy will be discussed.

Biomaterial scaffolds are very porous and are used to aid in tissue regeneration by serving as a template that will guide the regenerating tissues. For a scaffold to be successful in its purpose, it must be biocompatible with the host's oral environment, it must be biodegradable, it must have a mechanical property similar or identical to the anatomical structure it is being inserted in (must also be able to withstand the mechanical stresses of surgery), and it must have a proper architecture that allows cells and nutrients to penetrate through (porous) (O'Brien).

Growth factors are biological components that are heavily involved in cell signaling pathways. As specific genes are transcribed, the RNA must be translated to synthesize proteins that will lead to the growth of adjacent and/or non-adjacent cells. As growth factors bind to extracellular receptors, a cascade of effects occurs leading to an induction or inhibition of specific gene transcription.

Together, biomaterial scaffolds, growth factors, and cells all serve as variables in a formula for tissue regeneration, or as depicted in Figure 11, tissue engineering. For the purposes of this thesis, different scaffolds will be discussed, as well as the roles of growth factors in dental tissue regeneration.

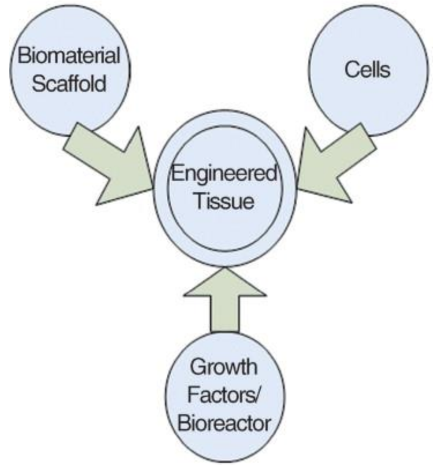


Figure 11. Image credit: O'Brien

A commonly-used biomaterial scaffold is demineralized dentin matrix (DDM). This scaffold is similar in structure to hydroxyapatite-tricalciumphosphate (HA/TCP). HA/TCP is an essential component of hard tissues in the body. DDM has also been used as a scaffold for bone regeneration using dental pulp stem cells (DPSCs). In a study that compared the efficacies of DDM and HA/TCP in the osteoinduction and osteoconduction of DPSCs, DDM was able to induce DPSCs into osteoblastic differentiation and produce bone. Furthermore, it was noted that DDM may also be effective in dentin regeneration (Kang, et al).

Another study aimed to determine whether scaffolds, copolymer of L-lactide and DL-lactive (PLDL), copolymer of DL-lactide (PDL), and HA/TCP would be effective in inducing DPSCs into odontogenic differentiation. After using RT-PCR to detect expression levels of DSPP, DMP1, MMP20, and PHEX, which is indicative of odontogenic activity, PLDL and PDL were confirmed to be promising scaffold candidates for dentin regeneration (Atalayin, et al).

Because biomaterial scaffolds have the possibility of increasing the risk of inflammation and infection, an approach that uses DPSCs as a 3-dimensional construct was developed. After combining the construct with hydrogel and placing it in the root canal of a human tooth, the tooth was implanted subcutaneously into immunocompromised mice. Following histological analyses, pulp-like tissues rich with blood vessels were observed, as well as odontoblast activity where the dental pulp makes contact with dentin. These results were indicative of regenerated dental structures within the tooth (Itoh, et al).

Growth factors, in dental tissues are responsible for mediation of tooth development. In oral development, IGF, TGF-β and FGF play major roles (Gilbert et al, Alkharobi et al, & Du, et al). The importance of growth factors in regenerative therapy is their ability to increase incidence of tissue regeneration. Currently, there are laser therapy methods that use lasers to increase levels of growth factors inducing stem cell differentiation, osseointegration, and bone healing. One study used a low-intensity laser at 904 nm on newly-loaded dental implants. The experimental ground exhibited profound bone healing and osseointegration (Mikhail, et al). Another study showed the low-power laser (LPL) photoactivation is a minimally-invasive method capable of regenerating dental tissues. Here, photoactivation lead to increased levels of TGF-β1, which induces odontoblastic differentiation in dental stem cells (Arany et al).

IV. Issues of Stem Cell-based Regenerative Therapy

The main factor that has limited the field of stem cell biology and stem cell research for clinical use is ethics. Ethical issues arise very often in healthcare. For years, the main ethical concern that many people had was from where stem cells were being isolated. Because stem cells are present in development, it has been thought that the best place to isolate stem cells from was from the womb of pregnant women. Scientifically-speaking, there is no issue. Ethically-speaking, there is a glaring issue. Stem cells have been able to be gathered from other regions of the body, but the best places are places where there is a lot of active development. Where this relates to dentistry is that stem cells have been acquired from baby deciduous teeth. While some critics may have their reservations about this as well, this has been much less of an issue. This is because every single human (ignoring pathological cases) has had deciduous teeth at some point in their life. Certain people, like pregnant women, will not have to be targeted. All that must be done here, is once the deciduous teeth have been shed, the teeth must be processed and the cells isolated (Amanda H).

Other issues of regenerative therapy are rejection and limited supply of specific stem cell types. As is the case with organ transplants, transplantation of stem cells originating from another individual with a different genotype, may result in rejection. In order to achieve the best results for dental regeneration, a specific type of stem cell may need to be used, but if the supply of that cell type is limited, the ability to go through with regenerative procedures is also limited.

The issue of limited stem cell supplies can be circumvented through induced pluripotency. Induced pluripotency is the concept of converting an adult, somatic cell (already differentiated) back to a pluripotent state as if it were a stem cell again. It was once thought that when a stem cell differentiates and becomes an adult somatic cell, there is no going back to a pluripotent state. Because all cells contain the entire organismal genome in their nuclei, exposing genes to the right transcription factors could possibly result in restored pluripotency. This feat was accomplished in 2006 by two Japanese researchers of Kyoto University. The researchers targeted multiple adult mouse somatic cells by inserting the Sox2, Oct4, c-Myc, and Klf4 genes. These genes code for the necessary transcription factors to induce pluripotency in somatic cells. Induced pluripotency may serve as the solution for the need of specific stem cells from a specific host (Gilbert, pgs. 328-329).

V. Future Implications for Dentistry

Commonplace Dental Regenerative Therapy

Regenerative therapy will likely become normalized in dentistry. Once it becomes widely known that teeth can finally be regrown after losing them, many dental offices could expect to see a large influx of patients. Regenerative therapy would be incorporated into regular treatment plans.

Today, chronic diseases plague the United States; as dentistry is concerned, dental caries is number one. When patients present with advanced tooth decay, the only feasible option is for the tooth to be extracted. The major, long-term problem of having teeth extracted and not having them replaced, is how the adjacent teeth will begin to shift. As a result of their shifting, the jaw bone will begin to lose mass, which will eventually lead to the loss of more teeth. Many times, patients opt for tooth implants, but if the patients do not have enough bone to support the implant, they will either require a bone graft, or they will just not receive the implant. The cases in which the patient does not have a tooth implanted, they may inquire about other dental prosthetic devices, such as dentures. The problem here, as previously stated, is that dentures are not ideal for a number of reasons. One of the main reasons being, dentures do not support the alveolar bone in the jaw, which will lead to continual bone resorption. The best fix for this would be for the patient to have the tooth extracted, and almost immediately, the tooth socket being injected with stem cells and their ideal growth environment. This way, a tooth will develop where the patient's original tooth once was.

As treatment plans are concerned, it comes down to what is the best clinical decision for the patient, what is the best economic decision for the patient, as well as what is even possible and feasible for the dentist to follow through. These are just a few factors that play into a patient's treatment plan. Depending on the case, it will just come down to whether the patient should receive a partial denture, a bridge, an implant, or a regenerated tooth.

There may even be instances where patients will simply inquire about regrown teeth and other structures. A possible issue that was mentioned earlier is the occurrence of patients that present with no major dental issues asking to have whichever teeth they do not like, removed, and having them replaced with regrown teeth. This would be an ethical issue of which dentists would have to be aware and know exactly how to address. These sort of occurrences would be the direct result of patients hearing about other people having teeth regrown, gums regrown, and other structures of the oral cavity, or even seeing something of the like on television.

Regenerative therapy in dentistry will someday be nothing of taboo. As it pertains to medicine, once upon a time, it was thought that cochlear implants would not even be possible. If a patient has hearing problems, and the traditional hearing aid is not of much benefit to them, there are no other options. But today, it is very common to see cochlear implants in children and adults alike. Someday, this will be the reality of regenerative dentistry. After more time has passed, it might not be even considered to be the results of state-of-the-art technology and methods.

Socioeconomic Considerations of Regenerative Dentistry

Another highly important aspect to be considered for dental regenerative therapy is whether or not it would even be affordable. And if it is affordable, which populations and demographics would be able to benefit from this form of therapy. In dentistry, it is of the utmost importance that patients receive the dental care they need. More often than not, the patients' ability to receive this care is contingent upon their ability to afford the care.

A major contributing factor in the treatment of dental patients is the presence or absence of dental insurance. As reported in a national children's health survey, as much as 22.1% of children in the United States lack dental insurance. Additionally, more than one-fourth of all children in the United States have routine preventative dental visits (Liu, et al). This is important to note, because many dental visits are related to dental caries, and most cases of dental caries are attributed to those of children. On top of this, it has also been shown that children living in rural areas were less likely to have dental insurance coverage, while being more likely to have unmet dental needs. Dental insurance disparities and dental care disparities are directly related to family socioeconomic status (Liu, et al).

It is expected for regenerative therapy to be on the more expensive end of the dental care spectrum, as so many parts go into therapy. Not only cell cultures being isolated, manipulated, and maintained, the necessary equipment for these processes must also be incorporated into patient treatment. Here, it would be very beneficial to have dental insurance, but for populations that have been shown to lack dental insurance, or simply, dental care, like minorities, unfortunately, regenerative therapy might not be much of an option (Liu, et al).

In relation to the presence or absence of dental insurance, the cost effectiveness of different dental procedures and treatments must also be considered when patients are deciding which treatment is best for them. From a pure physiological standpoint, regenerative therapy is the “best” objective treatment. From a pure economic standpoint, the opposite may be true for some patients. Because regenerative therapy in dentistry is a very new concept, there is no sufficient evidence of cost effectiveness for regenerative therapy. Additionally, there is not a profound amount of literature on the cost effectiveness of dental treatments and the comparison of different treatments. Here, the cost effectiveness of regenerative therapy will be compared to that of dental implants.

A study recently conducted in Japan, aimed to compare the cost effectiveness of treatments for missing first molars using dental implants versus fixed dental prostheses. As previously stated in this paper, dental implants are artificial teeth, comprising a titanium implant drilled into the upper and/or lower jaw bones, and capped with a crown. Fixed dental prostheses include irremovable prosthetic devices placed in the oral cavity, such as veneers, bridges, inlays and onlays, and crowns. The study used the General Oral Health Assessment Index (GOHAI) as an indicator of oral quality of life (QOL). Over the course of 30 years, patients treated with dental implants had a higher oral QOL value than patients treated with fixed dental prostheses (Korenori, et al).

The importance of regenerative therapy assuming the role of dental implants here when assessing cost effectiveness is that although dental implants may cost more, they will last must longer than fixed dental prostheses. The same is assumed for regenerative therapy. While regenerative treatment may be quite costly, especially without dental insurance, it is the more desirable option when considering longevity.

Feasibility of Regenerative Therapy

The feasibility of regenerative therapy in dentistry is reliant upon the patient's need for regenerative dentistry. This goes back to the common healthcare principle of finding the best treatment for a patient. Again, as part of the pledge that all practicing dentists have made, dentists must, first, do no harm (ADA). This must be applied to situations in which harm can be avoided.

If a patient receives veneers, but the underlying teeth begin to decay, indirectly, harm has been done. If a patient that is allergic to certain monomers present in acrylic resins receives dentures, and an allergic reaction occurs along with gum sores and residual bone loss, harm has been done (Rashid, et al). If a patient receives a dental implant, and the titanium posts leads to silent and systemic inflammation in the patient, harm has been done (Lechner, et al).

The purpose of this thesis is not to downplay the clinical benefits of various procedures, nor is its purpose to regard regenerative dentistry as the "end all, be all" in dental treatment. No treatment of any ailment of the human body is perfect, but if we are able to find a treatment that is as close to being "perfect," that should be our undertaking. Currently, in cancer research, we are actively searching for and developing ways to differentially and only target cancerous cells to treat cancer. In practical terms, there are cures for cancer, but are they perfect? Not at all. The ideal *cure* for cancer is a cure that can kill only cancer cells without harming normal cells. At this time in healthcare, there exists no such cure. This is concept is essentially what this thesis aims to touch in brief. If regenerative therapy can do little to no harm, especially when compared to current treatment options, further developing and implementing regenerative dentistry ought to be our priority. Although regenerative therapy may not be expected to be the best economic decision for some patients, helping patients and doing no harm is still the priority of all ethically-practicing dentists. It is for these reasons that regenerative therapy in dentistry is no only feasible, but a necessity.

Emergence of a New Dental Specialty

General dentists are capable of performing treatments and procedures related to all aspects of dentistry, but only to a certain extent. The specialties in dentistry all address the specific realms of dentistry that general dentists may not have the expertise in. As recognized by the American Dental Association (ADA), there are currently nine dental specialties. These include: endodontics; orthodontics; prosthodontics; periodontics; pediatric dentistry; dental public health; oral and maxillofacial pathology; oral and maxillofacial radiology; and oral maxillofacial surgery. These specialties can be categorized based upon common dental principles and procedures, such as root canals and tooth extractions. Each dental specialist will have a plethora of highly complex cases that require treatment plans with methodologies extending beyond the basic education that is acquired in dental school.

In a future where it has become commonplace to have stem cells used for regenerating the tissues and structures of the human dentition, the need for a new dental specialty could easily emerge. As more and more patients would opt to have their dental structures regrown rather than replaced with various prostheses, the necessity of a new dental specialty, regenerative dentistry, would become evident. As previously stated, there are nine specialties recognized by the ADA, but there are currently considered to be 13 dental specialties in total. (Brown, Dentistry Today).

Oral implantology, orofacial pain, dental anesthesia, and oral medicine are all newly-emerging dental specialties. While many dentists may be excited about these specialties, there is, however, much controversy surrounding the establishment of these four specialties. Just as the case for medical specialties, in order for a new dental specialty to be established, three main criteria must first be met: (1) the new specialty must be able to benefit the care of patients, and this must be determined based upon evidence, (2) the new specialty must be able to incorporate training and education programs for practitioners to become proficient in all aspects of said-specialty, and (3) the new specialty must be able to implement a "psychometric evaluation process" to test the degree to which the practitioners are, at the allowable minimum, competent in the specialty upon the conclusion of training and educational programs. (Siegel MA). These criteria, when they were appropriately argued, led to the amendment to section 5.H of the ADA Principles of Ethics and Code of Professional Conduct

According to the latest version of the American Dental Association's *Code of Ethics and Professional Conduct*, dentists are permitted to announce and market themselves to the public as specialists in any of the nine specialties recognized by the ADA or areas of dentistry that specialty recognition has been "granted under the standards required or recognized in the practitioner's jurisdiction, provided the dentist meets the educational requirements required for recognition as a specialist adopted by the American Dental Association or accepted in the jurisdiction in which they practice" (ADA). Until the court case "American Academy of Implant Dentistry v. Parker," and the amendments to section 5.H of the *Code of Ethics and Professional Conduct*, this was not ethically permitted for dentists to do.

In order for the ADA to officially recognize an area of dentistry as a specialty, a vote must be held. It is speculated by many that these votes (as it pertains to the four emerging specialties) are subject to undue bias. Some saw that the new specialties were not fit to be considered as official dental specialties, while others may have felt that certain new specialties may compete economically with existing specialties or general dentistry as a whole.

Furthermore, it was also heavily discussed that the ADA is merely a trade association. The entity that is responsible for dental specialty recognition are the state dental boards. It has historically been shown that the ADA is relied upon by the state boards for specialty recognition. This was similarly the case in the 1930's when there were only four American Medical Association-recognized medical specialties: ophthalmology, otolaryngology, obstetrics and gynecology, and dermatology and syphology. There were several emerging medical specialties that could not receive AMA recognition. As a result, these specialties came together to form the American Board of Medical Specialties (ABMS) a credentialing organization, and not a trade association (Siegel MA). Like the ADA, the AMA was a trade association. And like the ABMS, a specialty credentialing authority has been sought after. Ostensibly, the four emerging dental specialties, oral implantology, orofacial pain, dental anesthesia, and oral medicine, formed the American Board of Dental Specialties in 2015 (Brown RS). As was the case with medicine, it can be expected that the ABDS will lead the way in the establishment of more dental specialties in an effort to better treat patients. Currently, there are 20 medical specialties, and as recognized by the ABDS, there are 13 dental specialties. Based upon the developments mentioned in brief here, it would be interesting to see how regenerative dentistry could be formally established as a dental specialty.

Before regenerative dentistry is officially recognized as a dental specialty, it must first pass through all necessary stages of research and human clinical trials until the desired results (effectively and ethically regenerating teeth, periodontal tissues, and other dental structures) are achieved consistently and standardized in a multitude of clinical settings to the benefit of dental patients. Once this milestone has been reached, dentists and other officials working in regenerative dentistry must take the necessary steps to establish the necessary educational facilities and as part of established institutions. When set in place properly, dentists will be able to learn and practice procedures and methods of and relating to regenerative dentistry. Following this component specialty establishment, measures must be taken to implement psychometric testing protocols to test and measure the competency of the trained dentists. Based upon the credentials that realms of medicine and dentistry must meet in order to be recognized as specialties, and in going through with these measures, regenerative dentistry would hopefully be able to be officially established and recognized as a dental.

From here, many more patients would be able to receive the quality care that they deserve as humans. As previously stated, many of the current treatment methods currently employed in dentistry are not ideal for the true physiology of the human dentition and its associated systemic constituents. But, with the establishment and implementation of the specialty, regenerative dentistry, patients will now be able to have teeth and other structures of the oral cavity regrown; and hopefully, comparable to the full functional capability of their original teeth and structures.

Based upon all that regenerative dentistry entails, and its ability to meet the three criteria listed above, the field of dentistry could very likely see regenerative dentistry implemented as a newly-recognized dental specialty. By the time this occurs, oral implantolgy, orofacial pain, dental anesthesia, and oral medicine may already be fully-established and fully-recognized specialties.

VI. Conclusion

While this systematic review simply highlights the key aspects of regenerative therapy in dentistry, there are deeper concepts within that should be researched and discussed further by the scientific community. It is not only to the benefit of satisfying scientific curiosity, but research in stem cell-based regenerative therapy also serves to benefit future patients. Within healthcare, it is the duty of the practitioners to continue their education and develop and implement better techniques. As regenerative therapy is concerned, stem cell research is a major avenue. Stem cells provide fuel to the field of tissue engineering. Properly understanding the physiology, uses, and possible uses of stem cells may yield great results in regenerative therapy research, especially for regenerative dentistry. It is the goal for research to progress into a multitude of clinical trials, and then into standard practice.

This review has touched on the major aspects of research in regenerative dentistry that look very promising and should be further developed. Fully harnessing the capability and power of stem cells will change dentistry in a drastic way. To provide possible methods of implementing stem cell-based regenerative therapy in dentistry, several models of dental tissue regeneration have been described. Additionally, the mechanisms that lie behind regeneration have been mentioned.

Stem cell research is limited by different ethical considerations. In many cases, it is not the science of regeneration that is being questioned, but rather the morality of such practices that is being questioned. Fortunately, regenerative therapy in dentistry is able to avoid many of the ethical issue that have plagued stem cell research for so long. For instance, to regrow teeth and periodontal tissues, the stem cells of another host are not required. The patient’s stem cells can be harvested. This is done even easier when stem cells of deciduous teeth are isolated, cultured, and used. This is because, every single human being with any current or past dentition had deciduous teeth at some point.

Again, this thesis does not serve as a comprehensive review, but more so a review to highlight and outline where the strengths of regenerative research in dentistry lie and where further developments ought to be made. In this, economic impacts should also be considered. Following the full implementation of regenerative dentistry, dentistry will enter a realm of truly effective healthcare. When certain conditions present themselves, a new specialty may emerge as well. As long as there is a demand for such positive change, there ought to be a supply. Someday, conducting major research on regeneration of teeth and periodontal tissues with the use of stem cells will be something of the past, because regenerative dentistry will have been long established and commonplace. Patient care will be greatly improved, and that is the hope the current research methods.

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